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Impacts of algal blooms removal by chitosan-modified soils on zooplankton community in Taihu Lake, China

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Abstract

It is important to assess the effect on zooplankton when perform the environmental protection or restoration technology, especially removing algal blooms, because algae were the major primary producer in algal lakes. The influence on zooplankton community after half a year of algal blooms removed by chitosan-modified soils in Taihu Lake was assessed and the rationality of carrying out the process semiannually was evaluated in the present study. Morphological composition and genetic diversity of zooplankton community were investigated by microscope checkup and polymerase chain reaction-denatured gradient gel electrophoresis (PCR-DGGE). A total of 44 zooplankton taxa (23 protozoa, 17 rotifers, 3 copepoda and 1 cladocera) were detected by microscope checkup, and a total of 91 bands (28 bands amplified by primers F1427-GC and R1616, 63 bands amplified by primers Fung-GC and NS1) were detected by PCR-DGGE. The results of cluster analysis or detrended correspondence analysis indicated that there was no considerable difference in morphological composition of zooplankton and DGGE profiles between experimental and control sites, and DGGE profiles could represent the biologic diversity. The study showed that zooplankton community could recover original condition after half year of algal blooms removed by chitosan-modified soils and it was acceptable to apply this process semiannually. In addition, the results revealed that PCR-DGGE could be applied to investigate the impacts of the environmental protection or restoration engineering on zooplankton community diversity.

Key words: algal blooms; *Microcystis aeruginosa*; zooplankton community; chitosan-modified soils; Taihu Lake
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Introduction

It is well known that zooplankton play important roles in aquatic ecosystems in respect that they link primary producers and higher trophic levels. As crucial components of the aquatic food chain, the qualitative and quantitative changes in zooplankton population might bring a consequential impact on the whole aquatic ecosystem (Bianchi et al., 2003; Basima et al., 2006; Maazouzi et al., 2008; Medeiros and Arthington, 2008). Thus, the impact assessment of zooplankton community is important during conducting the environmental protection planning or restoration projects.

Taihu Lake is the third largest freshwater lake in China. It located in the central area of the Yangtze River Delta (30°55′40″N to 31°32′58″N, 119°52′32″E to 120°36′10″E). Recently, Taihu Lake is troubled by eutrophication and harmful algal blooms often occur between June and November. The average algal concentration was from 10^6 to 10^9 cells/L, and the concentration could be higher in many places in surface water (Pan et al., 2006a; Guo, 2007; Bai et al., 2009; Guan et al., 2009). The treatments of eutrophication and algal blooms were carried out subsequently, and removing algal blooms by clays was recommended as a promising and environmentally friend way to remove algal blooms (Anderson et al., 1997; Sengco and Anderson, 2004; Pan et al., 2006a). Generally, it is considered that the inter-particle forces and the hydrodynamics play pivotal roles in flocculation. The size, density, shape, surface charge and chemical compositions of clay particles and algal cells also affect their flocculation efficiency because of the contribution to the inter-particle forces, as well as to the collision and movement between algal cells and clay particles (Han and Kim, 2001; Pan et al., 2006a). Pan et al. (2006b) pointed out that chitosan modified solids can remove algal cells more effectively in freshwater, while the behavior of normal clay flocculation is more effective in salty waters.

Most of studies on impacts of removing harmful algal

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blooms on aquatic organisms focus on the toxicity of algaecide, impacts of aquatic physical and chemical properties changed aquatic organisms, and poisoning effect on aquatic organisms of intracellular brevetoxins released following treatment (Sengco and Anderson, 2004; Pierce et al., 2004; Anderson, 2009). However, as major primary producers, impact of algal blooms removal on high trophic levels is important during treatment. Even the process of algal blooms removal by chitosan-modified soils unaffected the growth of Local mussels *Cristaria plicata* (Leach) and submerged macrophytes *Myriophyllum spicatum* (Zou et al., 2006), as well as Yan et al. (2009) proved neither chemical conditions nor plankton succession are significantly affected by the algal removal process in artificial systems, the effect on natural freshwater ecology is still unclearly after this process. A comparison of zooplankton community between pre and post algal blooms removal (July 21, 2008 and August 22, 2008, respectively) showed that there was significant diversity (paired samples test, $p = 0.035$, the result unpublished). However, it was not sure whether difference existed post a longer time of the process. Therefore, in the present study, the zooplankton community collected in the experimental area and control area after half year of algal blooms removal was employed to appraise the impact by morphological composition and genetic diversity analysis. In addition, based on the evaluation, the frequency of algal blooms removal by chitosan-modified soils could be appraised synchronously.

1 Materials and methods

1.1 Collection and processing of samples

The samples were collected from eight sampling sites (six experimental sites and two control sites) at the enclosures located in the northern part of Taihu Lake on February 28, 2009 (Fig. 1). The algal blooms remove was conducted by chitosan-modified soils in experimental area in August, 2008. The composition of chitosan-modified soils and the methods of algal blooms removal were described by Pan et al. (2006a) and Yan et al. (2009). Planktonic organisms for qualitative and quantitative analysis were collected and pretreated according to Yan et al. (2007). Briefly, samples for qualitative analyses of plankton were collected via horizontal surface tows with a No.25 plankton net and preserved in 4% formalin solution. Live plankton samples were collected to confirm the taxonomic status of certain species, synchronously. Equal volumes of surface and bottom water were sampled and mixed for each samples for quantitative analysis of dominant protozoa and rotifers and genetic diversity analysis of eukaryotic zooplankton. Approximately 500 mL samples for quantitative analysis were fixed with Lugol's solution after collection with a polypropylene bucket, sedimented for 24 hr and subsequently concentrated to 30 mL. Approximately 500 mL of each sample was filtered by a glass-fiber (GF/C) for extracting community genomic DNA .

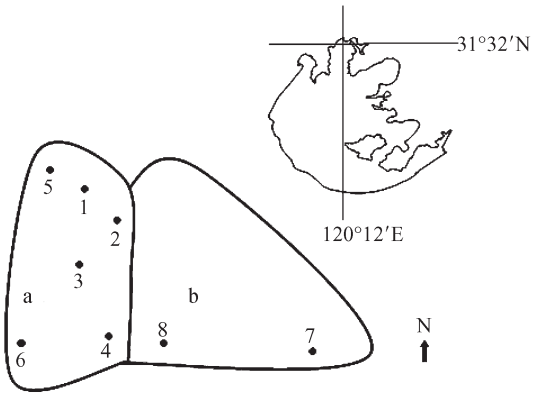

Fig. 1 Map of sampling sites in Taihu Lake, China. (a) experimental area is approximately 5000 m^2; (b) control area is approximately 10,000 m^2.

1.2 Physicochemical factors analysis and morphological analysis of plankton

Physicochemical factors, including pH, transparency, dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP), NH_4^+-N, NO_3^--N, PO_4^{3-}-P and chlorophyll-*a* were determined according to standard methods (Huang, 2000). Planktonic organisms were examined and counted using an Axioplan 2 Imaging microscope (Ziess, Jena, Germany) according to previous studies (Chiang and Du, 1979; Shen et al., 1990; Wang et al., 1961; Zhang and Huang, 1991; Research Group of Carcinology, 1979). For quantitative analyzing of dominant rotifer, the organisms in 1 mL concentrated samples collected from each sampling site were examined and counted. The organisms in 0.1 mL concentrated samples were examined and counted for quantitative analyzing of dominant protozoa.

1.3 DNA extraction and PCR amplification

The community genomic DNA was extracted using the method described by Yan et al. (2007) with some modifications. The filters were cut into small scraps and then transitorily commixed in 3 mL lyses solution (10 mmol/L Tris-Cl, 0.5% SDS, 100 mmol/L EDTA and 0.1 mg/ml proteinase K), subsequently incubated in 55°C bath for 12 hr. After being centrifuged with 10,000 ×*g* (10 min) at room temperature, the upper liquid was transferred into a new sterile tube and ultimately applied with phenol-chloroform purification method. Eukaryotic 18S rRNA genes were amplified applying two different sets of primers (Table 1). Each PCR reaction mixture (25 μL) contained 1× PCR buffer, 2 mmol/L $MgCl_2$, 1.5 U Taq DNA polymerase, 80 μmol/L deoxynucleotide (Fermentas Inc., Hanover, USA), 0.5 μmol/L of each primer, and approximately 10 ng of template. PCRs were performed on a S1000™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA) with the conditions showed in Table 1. Part PCR products (4 μL) were detected by conventional electrophoresis in 1.4% (*W/V*) agarose gel with TAE 1× buffer, stained with ethidium bromide 0.5 μg/mL in TAE 1 × buffer. Other products were stored at –20°C for denaturing gradient gel electrophoresis (DGGE) analysis.

Table 1 Primers sequences and PCR conditions applying for touchdown PCR

Primers	Sequences (5′–3′)	PCR conditions	References
F1427-GC	GC clamp-TCTGTGATGCCCT-TAGATGTTCTGGG	An initial denaturation at 94°C for 5 min and 10 touchdown cycles of denaturation at 94°C for 30 sec, annealing at 68°C (with the temperature decreasing 1°C per cycle) for 30 sec, and extension at 72°C for 1 min, then followed 30 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min. Finally, the extension at 72°C for 10 min.	Yan et al., 2007
R1616	GCGGTGTGTACAAAGGGCA-GGG		
Fung-GC	GC clamp-ATTCCCCGTTACC-CGTTG	An initial denaturation at 94°C for 5 min and followed 32 cycles of 94°C for 30 sec, 52°C for 30 sec and 68°C for 1 min. Finally, the extension at 68°C for 5 min.	Suzuki et al., 2009
NS1	GTAGTCATATGCTTGTCTC		

GC clamp: 5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-3′.

1.4 DGGE analysis

DGGE was performed with an INGENYphorU-2 system (INGENY International BV, Leiden, The Netherlands) to detect the genetic diversity of eukaryotes between experimental area and control area. For analysis of the products amplified using primer F1427-GC and R1616, 8% (*W/V*) polyacrylamide (acrylamide:bisacrylamide = 37.5:1) gels were cast with a denaturing gradient ranging from 30% to 50% and ran at 120 V for 12 hr in 1× TAE buffer (40 mmol/L Tris acetate, 1.0 mmol/L EDTA and 40 mmol/L acetic acid, pH 7.6) at 60°C. Approximately 600 ng of PCR product was loaded for each lane. For analysis of the products amplified using primer Fung-GC and NS1, 8% polyacrylamide gels were cast with a denaturing gradient ranging from 15% to 40% and ran at 120 V for 12 hr in 1 × TAE buffer at 60°C. Approximately 600 ng of PCR product was loaded for each lane. After electrophoresis, the gels were stained with 1 × SYBR Gold (Molecular Probes Europe BV, Leiden, The Netherlands) for 30 min, and then photographed with a UVP Imaging System (UVP Inc., USA). The gel images were further processed using Adobe Photoshop 8.0.1 to maximize image contrast before analyzed using Quantity One 4.6.2 software (Bio-Rad Laboratories, Inc., USA).

1.5 Data processing

The DGGE profiles and morphological analysis of zooplankton were analyzed using cluster analysis to determine differences in the eukaryotic microbial communities among experimental area and control area. The analyses were carried out with Quantity One 4.6.2 software and NTSYS 2.10 software. Cluster analysis determining the quantitative differences in the protozoa and rotifers community were carried out with SPSS 13.0 software. Detrended correspondence analysis (DCA) was used for ordination analysis of data obtained from the quantitative analysis of the protozoa and rotifers community. The others were analyzed by SPSS 13.0 software.

2 Results

2.1 Physicochemical context and morphological composition

Figure 2 compares the physicochemical conditions between experimental sites and control sites. It was clearly showed that the concentrations of NO_3^--N and chlorophyll-*a* were reduced significantly, and the concentrations of DO and TN were also reduced. However, the concentrations of TP, NH_4^+-N and PO_4^{3-}-P were increased significantly. The result did not indicate the improvement of transparency. The unweighted pair-group method with arithmetic means (UPGMA) clustering indicated that physicochemical conditions between experimental sites and control sites were generally differentiated (Fig. 3f).

A total of 44 zooplankton taxa (23 protozoa, 17 rotifers, 3 copepoda and 1 cladocera) and 18 algal taxa were detected, and 9 taxa of zooplankton were detected at all sites and 14 taxa were restricted to single sites (Tables 2 and 3). Site 8 was detected the most taxa (24 taxa), followed by site 4 (22 taxa) and site 7 (21 taxa). And site 3 was detected the least taxa (17 taxa), followed by site 1, site 2 and site 6 (19 taxa). UPGMA clustering based on the algae, protozoa, rotifers, copepoda and cladocera and zooplankton showed there was no significant difference between experimental sites (sites 1 to 6) and control sites (sites 7 and 8). There were different cluster patterns between algae, protozoa, rotifers, copepoda and cladocera, and all of zooplankton, but site 7 was not clustered a branch with site 8 in all patterns (Fig. 3). This showed that the zooplankton and algal community structures could recover original condition after half a year of the processing and the differences of zooplankton community structures within experimental sites covered those between experimental sites and control sites.

There were 7 relative dominant protozoa taxa and 11 relative dominant rotifer taxa detected in eight sites by quantitative analysis. Site 7 was detected the most taxa and site 1 was detected the least taxa of protozoa. While, site 3 was detected the most taxa and site 4 was detected the least taxa of rotifers. UPGMA clustering and DCA ordination based on quantitative composition of dominant protozoa showed a slender difference between experimental sites (sites 1 to 6) and control sites (sites 7 and 8) in reigning protozoa, despite site 5 which did not cluster a branch with other experimental sites, but clustered with control sites (Fig. 4a). However, no significant difference between experimental sites (sites 1 to 6) and control sites (sites 7 and 8) in reigning rotifers quantitative results was observed (Fig. 4b).

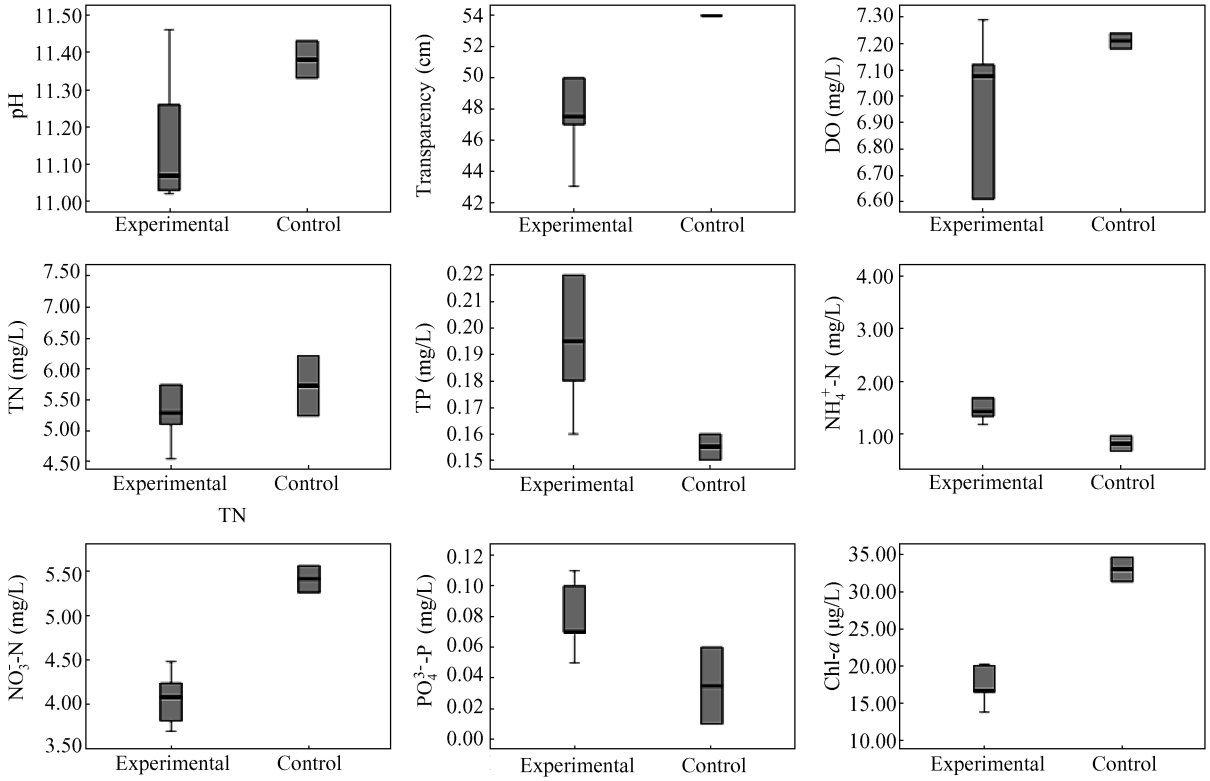

Fig. 2 Comparison of physicochemical conditions between experimental sites and control sites. Low margins, middle lines and upper margins of boxplots indicate 5, 50 and 95 percentiles, respectively.

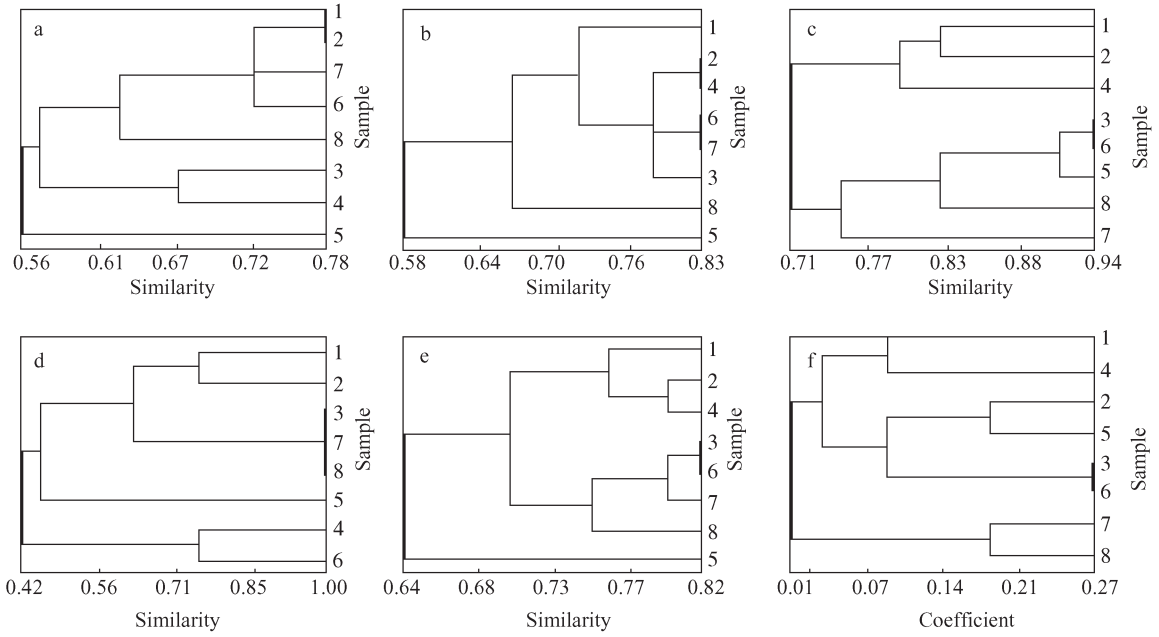

Fig. 3 Group relationship of the eight sampling sites on the basis of qualitative results and physicochemical factors. UPGMA clustering based on the qualitative composition of algae (a), protozoa (b), rotifers (c), copepoda and cladocera (d) and all of zooplankton (e), and quantitative analysis of physicochemical factors (f). Samples 1–6 were collected at experimental area that carried out algal blooms removal by chitosan-modified soils in August, 2008 and samples 7 and 8 were collected at control area.

2.2 Plankton community structure depicted by DGGE profiles

Based on the primers F1427-GC and R1616, a total of 28 bands were found, including 13 bands detected at all sites and 2 bands detected at single site. Site 6 was detected the most bands (24 bands), followed by site 7 and site 8 (23 bands), and site 2 and site 3 were detected the least (20 bands). Based on the primers Fung-GC and NS1, a total of 63 bands included 23 bands detected at all sites and 5

Table 2 Qualitative results of algae from eight sampling sites

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Asterionella sp.	+	+	+	+	+	+	+	+
Chlamydomonas sp.	+	+	+	+	+	+	+	+
Cryptomonas sp.			+	+	+		+	
Dinobryon sp.	+	+			+			
Eudorina elegans					+			
Euglena sp. 1	+							+
Euglena sp. 2[a]			+					
Euglena acus		+		+	+			
Euglena vividis	+	+		+			+	
Euglena oxyuris			+		+			
Gymnodinium sp.			+	+				+
Pandorina sp.				+				+
Perenema sp.								+
Peridinium sp.			+		+	+		
Phacus anomalus	+		+	+				
Phacus pyrum	+		+	+		+		
Synura sp.	+	+	+	+	+	+	+	+
Trachelomonas sp.			+	+			+	

[a] Another species of genus *Euglena* which different from *Euglena* sp. 1.

bands detected at single site. Site 4 and site 6 were detected the most bands (47 bands), followed by sites 7 and 8 (41 bands). Site 1 was detected the least (27 bands), followed by sites 2 and 3 (35 bands and 33 bands, respectively). The UPGMA clustering based on the DGGE profiles showed there was no significant difference between experimental sites (1 to 6) and control sites (7 and 8) (Fig. 5). There was no considerable difference in profiles among sites. Even some bands were widespread, occurring in overall sites, most bands were restricted to one or a few sites.

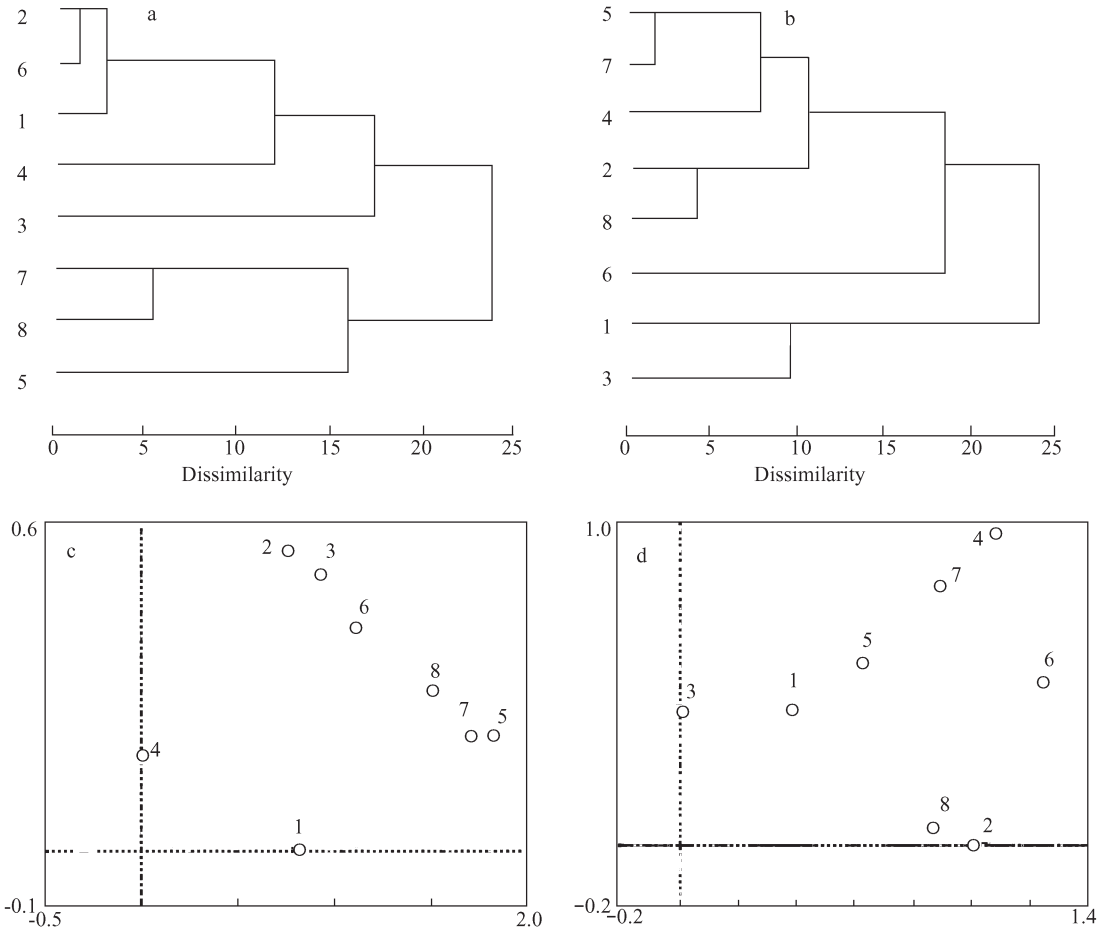

Fig. 4 Protozoa and rotifers community relationships of the eight sampling sites on the basis of quantitative results. Between – group Linkage clustering of the protozoa (a) and rotifers (b) based on the dominion quantitative composition. DCA ordination of the protozoa (c) and rotifers (d) based on the dominions quantitative composition. Samples 1–6 were collected at experimental area that carried out algal blooms removal by chitosan-modified soils in August, 2008 and samples 7 and 8 were collected at control area.

Table 3 Qualitative results of plankton from eight sampling sites

Taxa		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Protozoa	*Carchesium* sp.	+				+			
	Centropyxis aculeata					+	+		
	Coleps hirtus	+		+	+		+	+	+
	Cyclidium sp.			+					
	Cyclidium versatle								+
	Codosiga umbellata				+				
	Colpidium sp.							+	+
	Difflugia sp.	+							
	Difflugia gramen						+		
	Difflugia fallax					+			
	Hastatella radians								+
	Monas minima	+	+	+	+	+	+	+	+
	Monas socialis			+	+				+
	Monosiga robusta	+				+			
	Paramecium caudatum		+		+	+	+	+	+
	Pleuromonas jaculans	+	+		+				
	Raphidiophrys sp.	+	+	+	+	+	+	+	+
	Strobilidium sp.								+
	Holophrya atra				+				+
	Tintinnopsis wangi	+	+	+	+	+	+	+	+
	Vorticella companulla		+			+			
	Vorticella convallaria	+	+	+	+	+	+	+	+
	Vorticella microstoma					+		+	
Rotifers	*Asplanchna girodi*				+				
	Brachionus angularis		+	+	+	+	+	+	+
	Brachionus calyciflourus	+	+	+	+	+	+	+	+
	Brachionus quadridentatus								+
	Cephalodella sp.				+	+	+		
	Filinia sp.		+						
	Filinia maior	+						+	
	Filinia iongiseta			+		+	+	+	+
	Keratella cochlearis	+	+	+	+	+	+	+	+
	Keratella quadrata	+	+	+	+	+	+	+	+
	Keratella valga							+	
	Monostyla sp.							+	
	Polyarthra dotichoptera	+	+	+	+	+	+	+	+
	Polyarthra trigla	+	+	+	+	+	+	+	+
	Rotaria citrina	+	+	+	+		+	+	+
	Synchaeta sp.	+	+		+				
	Trichocerca sp.								+
Copepoda[a]	Calanoida	+	+		+				
	Cyclopoida		+	+				+	+
	Nauplius [b]	+	+	+	+		+	+	+
Cladocera	*Chydorus sphaericus*				+		+		

[a] Copepoda was differentiated to order; [b] nauplius was considered as one taxon in present study.

3 Discussion

Zooplankton play significant roles in linking primary producers and higher trophic levels in aquatic ecosystems (Yan et al., 2007; Maazouzi et al., 2008; Medeiros and Arthington, 2008). Thus it is important to assess the impact of performing environmental protection or restoration technology on zooplankton. It has been reported that the process changes the physical and chemical properties (e.g,. water transparency, phosphate concentration) when removing algal blooms by chitosan-modified soils (Pan et al., 2006b; Zhang et al., 2007), and the comparison of zooplankton community between pre and post algal blooms removal showed significant diversity (paired samples test, $p = 0.035$, the result unpublished). However, our result from morphological composition and genetic diversity analysis indicated that it did not change the zooplankton community. The reasons may that algae have not been removed completely by chitosan-modified soils as the limit of removal efficiency (Pan et al., 2006b), and the algal diversity been recovered original condition after half year of algal blooms removal.

Although the diversity of zooplankton and algae recovered original condition after half a year of algal blooms removed, the chlorophyll-*a* concentration of experimental sites (17.30 ± 1.01 μg/L) were still significant less than those of control sites (33.04 ± 1.67 μg/L) and there was obvious diversity of physicochemical factors between experimental group (site 1 to site 6) and control group (site 7 and site 8) (Figs. 2 and 3f). It was shown that the diversity of physicochemical factors caused by the process did not bring the negative impact on zooplankton post the process semiannually. Thus, the algal blooms removal by chitosan-modified soils could not bring an accumulative negative effect on zooplankton when the process was carried out semiannually.

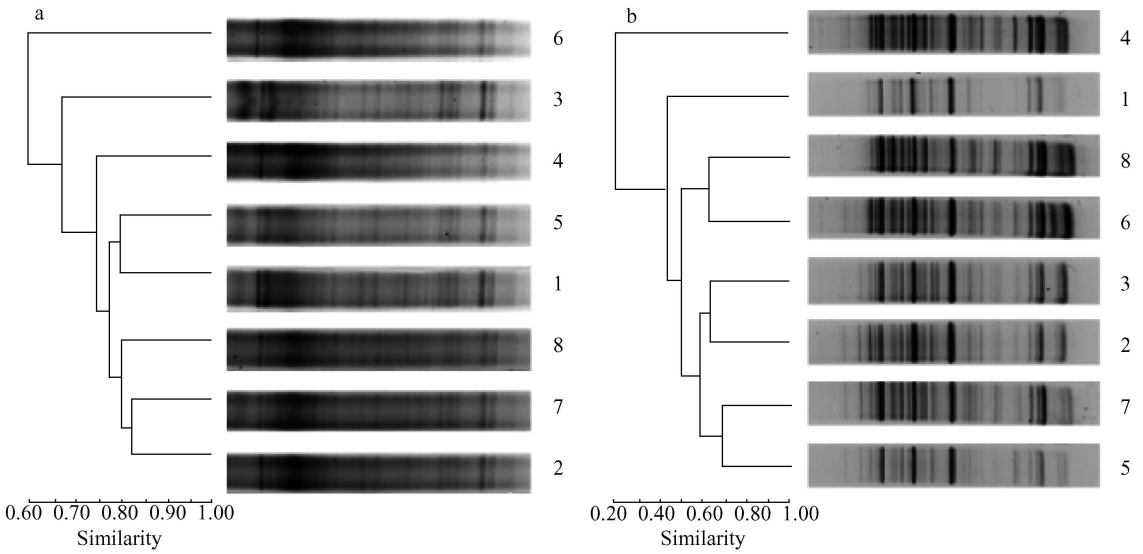

Fig. 5 UPGMA clustering of DGGE profiles obtained with eukaryotic primers F1427-GC and R1616 (a), and Fung-GC and NS1 (b). Samples 1–6 were collected at experimental area that carried out algal blooms removal by chitosan-modified soils in August, 2008 and samples 7 and 8 were collected at control area.

The reigning protozoa quantitative result showed a small difference between experimental sites (site 1 to site 6) and control sites (site 7 and site 8) (Fig. 4a), while it did not showed difference in the higher trophic level, reigning rotifer. The reason may be that the decrease of algal quantity following algal blooms removal brought on the change of reigning protozoa quantity. However, as the self regulation of aquatic ecosystems through food webs, the decrease of algal quantity could not impact the quantity of rotifer.

The correlation between the bands detected based on the primers F1427-GC and R1616, and the primers Fung-GC and NS1 was significant at the 0.05 level (N = 7, r = 0.771, p = 0.043) except site 4, suggesting that the biologic diversity represented by PCR-DGGE and PCR-DGGE could be applied to investigate the impacts of the environmental protection or restoration engineering on zooplankton community diversity. Site 4 was detected the most bands on DGGE profiles based on the primers Fung-GC and NS1, but detected almost the least bands based on the primers F1427-GC and R1616, this perhaps caused by some bands detected based on that the primers F1427-GC and R1616 were neglected as those were not very clear and the difference of the bands detected based on the primers F142-GC and R1616 was minor (only 2 or 3 bands), so that the neglect of unclear bands could affect the result.

The detected band numbers were different between the profiles based on the primers F1427-GC and R1616 and the profiles based on the primers Fung-GC and NS1. The reason may be different DNA segments amplified by PCR based on that different primer pairs had different mutation rates (Woese, 1987), thus produced different DNA fingerprints on DGGE profiles. However, the clustering result based on the DGGE profiles obtained with the two pairs of primers both do not have significant difference of zooplankton between the experimental area and the control area.

4 Conclusions

The zooplankton community could recover original condition after half year of algal blooms removal using chitosan-modified soils and it was acceptable that the process was carried out semiannually. In addition, PCR-DGGE could be applied to investigate the impacts of the environmental protection or restoration technologies on zooplankton community diversity. Considering the limitation of that only one investigation could not show all impact on the zooplankton during the period of long-term algal blooms removal using chitosan-modified soils, long-term investigation about the effect of this process will be performed.

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